

Claims:

1. **(Currently amended)** A method for mass spectrometric analysis of a 100uL sample comprising or suspected of comprising one or more thyroid hormones, the method ~~comprising~~ consisting of the steps of:
 - (a) providing a sample comprising or suspected of comprising one or more thyroid hormones, wherein the sample is approximately 100 μ L;
 - (b) deproteinating the sample;
 - (c) separating the one or more thyroid hormones from the sample; and
 - (d) analyzing the one or more thyroid hormones using a mass spectrometer, wherein the analysis does not involve an extraction step comprising evaporation and redissolving the extracted thyroid hormone, and the analysis is done in less than an hour.
2. **(Original)** The method according to claim 1 wherein the one or more thyroid hormones are selected from the group consisting of T3 and T4.
3. **(Previously presented)** The method according to claim 1 wherein the sample comprising or suspected of comprising one or more thyroid hormones is obtained from a biological sample selected from the group consisting of blood, plasma, serum, urine and saliva.
4. **(Original)** The method of claim 3 wherein the biological sample is blood.
5. **(Original)** The method of claim 3 wherein the biological sample is plasma.
6. **(Original)** The method of claim 3 wherein the biological sample is serum.
7. **(Original)** The method of claim 3 wherein the biological sample is urine.
8. **(Original)** The method of claim 3 wherein the biological sample is saliva.

9. **(Canceled)** The method according to claim 1 wherein size of said sample containing one or more thyroid hormones is at least about 100 μ L.
10. **(Original)** The method according to claim 1 wherein said step of deproteinating the sample comprises:
 - (a) adding acetonitrile, containing internal standards;
 - (b) vortexing the sample; and
 - (c) subjecting the sample to centrifugation.
11. **(Original)** The method according to claim 1 wherein said step of deproteinating the sample comprises subjecting the sample to precipitation with an agent containing internal standards, said agent selected from the group consisting of methanol, ethanol and salt.
12. **(Previously presented)** The method according to claim 1 wherein said step of separating the one or more thyroid hormones from the sample comprises introducing the sample to a liquid chromatography apparatus comprising a column and subsequently eluting the thyroid hormones from the column.
13. **(Original)** The method according to claim 12 wherein said step of separating the one or more thyroid hormones from the sample comprises the use of a C-18 column.
14. **(Original)** The method according to claim 1 wherein said step of separating the one or more thyroid hormones from the sample comprises the use of a combined liquid chromatography spectrometry apparatus.
15. **(Original)** The method according to claim 14 wherein the one or more thyroid hormones are introduced into a mass spectrometer directly after being separated from the sample by way of an on-line extraction and use of a built-in switch valve.

16. **(Original)** The method according to claim 1 wherein the mass spectrometer is a liquid chromatography-tandem-mass spectrometer.
17. **(Original)** The method according to claim 16 wherein the liquid chromatography-tandem mass spectrometer is equipped with an electrospray ionization source.
18. **(Original)** The method according to claim 1 wherein said step of analyzing the one or more thyroid hormones using a mass spectrometer comprises an ionization technique selected from the group consisting of photoionization, electrospray ionization, atmospheric pressure chemical ionization, and electron capture ionization.
19. **(Original)** The method according to claim 18 wherein said ionization technique is electrospray ionization.
20. **(Original)** The method according to claim 18 wherein said ionization is performed in positive mode.
21. **(Original)** The method according to claim 18 wherein said ionization is performed in negative mode.
22. **(Original)** The method according to claim 1 wherein said step of analyzing the one or more thyroid hormones using a mass spectrometer comprises multiple reaction monitoring.
23. **(Original)** The method according to claim 1 wherein said step of analyzing the one or more thyroid hormones using a mass spectrometer comprises selected ion monitoring.
24. **(Original)** The method according to claim 1 wherein the sample comprises a plurality of thyroid hormones and they are analyzed simultaneously.
25. **(Original)** The method according to claim 1 wherein the sample comprises a plurality of thyroid hormones and they are analyzed sequentially.

26. **(Original)** The method according to claim 1 wherein the sample is analyzed by isotope dilution tandem mass spectrometry.
27. **(Previously presented)** A method of instructing an analysis of a sample comprising or suspected of comprising one or more thyroid hormones, the method comprising providing instructions to prepare the sample according to steps (b) and (c) of claim 1 and analyze the one or more thyroid hormones from the sample according to step (d) of claim 1.
28. **(Currently amended)** A method for mass spectrometric analysis of a 700uL sample comprising or suspected of comprising one or more thyroid hormones and one or more steroid hormones, ~~comprising~~ consisting of the steps:
- (a) providing a sample containing one or more thyroid hormones and one or more steroid hormones, wherein the sample is approximately 700uL;
 - (b) deproteinating the sample;
 - (c) separating the one or more thyroid hormones and the one or more steroid hormones from the sample; and
 - (d) analyzing the one or more thyroid hormones and the one or more steroid hormones using a mass spectrometer,
- wherein the analysis does not involve an extraction step comprising evaporation and redissolving the extracted thyroid hormone, and the analysis is done in less than an hour.
29. **(Original)** The method according to claim 28 wherein the thyroid hormones are selected from the group consisting of T3 and T4.
30. **(Original)** The method according to claim 28 wherein the steroid hormones are selected from the group consisting of Dehydroepiandrosterone (DHEA), Dehydroepiandrosterone sulphate (DHEAS), Aldosterone, Cortisol, 11-Deoxycortisol, Androstenedione, Testosterone, Estradiol, 17-OH Progesterone,

Progesterone, Allopregnanolone, 16-OH Estrone, 2-OH Estrone, Estrone, and Estriol.

31. **(Original)** The method according to claim 28 wherein the sample is obtained from a biological sample selected from the group consisting of blood, plasma, serum, urine and saliva.
32. **(Original)** The method of claim 31 wherein the biological sample is blood.
33. **(Original)** The method of claim 31 wherein the biological sample is plasma.
34. **(Original)** The method of claim 31 wherein the biological sample is serum.
35. **(Original)** The method of claim 31 wherein the biological sample is urine.
36. **(Original)** The method of claim 31 wherein the biological sample is saliva.
37. **(Canceled)** The method according to claim 28 wherein size of said sample containing one or more thyroid hormones is at least about 100 μ L.
38. **(Canceled)** The method according to claim 28 wherein size of said sample containing one or more steroid hormones is at least about 700 μ L.
39. **(Original)** The method according to claim 28 wherein said step of deproteinating the sample comprises:
 - (a) adding acetonitrile, containing internal standards;
 - (b) vortexing the sample; and
 - (c) subjecting the sample to centrifugation.
40. **(Original)** The method according to claim 28 wherein said step of deproteinating the sample comprises subjecting the sample to precipitation with an agent containing internal standards, said agent selected from the group consisting of methanol, ethanol and salt.

41. **(Previously presented)** The method according to claim 28 wherein said step of separating the one or more thyroid hormones and the one or more steroid hormones from the sample comprises introducing the sample to a liquid chromatography apparatus comprising a column and subsequently eluting the hormones from the column.
42. **(Original)** The method according to claim 41 wherein said step of separating the one or more thyroid hormones and the one or more steroid hormones from the sample comprises the use of a C-18 column.
43. **(Original)** The method according to claim 28 wherein said step of separating the one or more thyroid hormones and the one or more steroid hormones from the sample comprises the use of a combined liquid chromatography spectrometry apparatus.
44. **(Original)** The method according to claim 43 wherein the one or more thyroid hormones and the one or more steroid hormones are introduced into the mass spectrometer directly after being separated from the sample by way of an on-line extraction and use of a built-in switch valve.
45. **(Original)** The method according to claim 28 wherein the mass spectrometer is a liquid chromatography-tandem-mass spectrometer.
46. **(Original)** The method according to claim 45 wherein the liquid chromatography-tandem mass spectrometer is equipped with an atmospheric pressure ionization source.
47. **(Original)** The method according to claim 45 wherein the liquid chromatography-tandem mass spectrometer is equipped with an electrospray ionization source.
48. **(Original)** The method according to claim 28 wherein said step of analyzing the one or more thyroid hormones and the one or more steroid hormones using a mass spectrometer comprises an ionization technique selected from the group

consisting of photoionization, electrospray ionization, atmospheric pressure chemical ionization, and electron capture ionization.

49. **(Original)** The method according to claim 48 wherein said ionization is performed in positive mode.
50. **(Original)** The method according to claim 48 wherein said ionization is performed in negative mode.
51. **(Original)** The method according to claim 48 wherein said ionization is performed in positive mode or negative mode.
52. **(Original)** The method according to claim 28 wherein said step of analyzing the one or more thyroid hormones and the one or more steroid hormones using a mass spectrometer comprises multiple reaction monitoring.
53. **(Original)** The method according to claim 28 wherein said step of analyzing the one or more thyroid hormones and the one or more steroid hormones using a mass spectrometer comprises selected ion monitoring.
54. **(Canceled)** The method according to claim 28 wherein the sample comprises a plurality of thyroid hormones and a plurality of steroid hormones, and they are analyzed simultaneously.
55. **(Original)** The method according to claim 28 wherein the sample comprises a plurality of thyroid hormones and a plurality of steroid hormones, and they are analyzed sequentially.
56. **(Original)** The method according to claim 28 wherein the sample is analyzed by isotope dilution tandem mass spectrometry.
57. **(Previously presented)** A method of instructing an analysis of a sample comprising or suspected of comprising one or more thyroid hormones and one or more steroid hormones, the method comprising providing instructions to prepare the sample according to steps (b) and (c) of claim 28 and analyze the

one or more thyroid hormones and the one or more steroid hormones from the sample according to step (d) of claim 28.

58. **(Previously presented)** A system for the mass spectrometric analysis of a sample comprising or suspected of comprising one or more thyroid hormones, and the sample is approximately 100 μ L comprising:
- (a) reagents for deproteinating the sample, including internal standards;
 - (b) reagents for analyzing the sample comprising or suspected of comprising one or more thyroid hormones using a mass spectrometer; and
 - (c) a mass spectrometer.
59. **(Original)** The system according to claim 58 wherein the mass spectrometer is a liquid chromatography-tandem mass spectrometer.
60. **(Currently amended)** A kit for use in mass spectrometric analysis of a sample comprising or suspected of comprising one or more thyroid hormones, and wherein the sample is approximately 100 μ L comprising:
- (a) reagents for deproteinating the sample comprising or suspected of comprising one or more thyroid hormones, including internal standards;
 - (b) reagents for separating the one or more thyroid hormones from the sample comprising or suspected of comprising one or more thyroid hormones;
 - (c) reagents for analyzing the one or more thyroid hormones using a mass spectrometer;
 - (d) a solution of one or more thyroid hormones; and
 - (e) instructions for analyzing the one or more thyroid hormones using a mass spectrometer,

wherein the analysis does not involve an extraction step comprising evaporation and redissolving the extracted thyroid hormone, and the analysis is done in less than an hour.

61. **(Original)** The kit according to claim 60 further comprising:

- (a) mobile phase solutions;
- (b) a chromatography column; and
- (c) a quality control specimen.

62. **(Currently amended)** Use of a mass spectrometer for sequentially or simultaneously analyzing a 100uL sample comprising or suspected of comprising a plurality of thyroid hormones, wherein the use consists of the steps of:

- (a) providing a sample comprising or suspected of comprising a plurality of thyroid hormone, wherein the sample is approximately 100 μ L;
- (b) deproteinating the sample;
- (c) separating the plurality of thyroid hormones from the sample; and
- (d) analyzing the plurality of thyroid hormones using a mass spectrometer,

wherein the analysis does not involve an extraction step comprising evaporation and redissolving the extracted thyroid hormone, and the analysis is done in less than an hour.

63. **(Original)** The use according to claim 62 wherein the mass spectrometer is a liquid chromatography-tandem mass spectrometer.

64. **(Previously presented)** The method of any one of claims 1 or 28 wherein the mass spectrometer is an API 2000™ mass spectrometer.

65. **(Previously presented)** The method of any one of claims 1 or 28 wherein the mass spectrometer is an API 3000™ mass spectrometer.